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Neutralization of interleukin-1β activity *in vivo* with a monoclonal antibody alleviates collagen-induced arthritis in DBA/1 mice and prevents the associated acute-phase response

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ABSTRACT. *Interleukin-1 (IL-1) has been implicated in the development and progression of a variety of acute and chronic inflammatory diseases. Due to its pro-inflammatory and tissue-degrading activities, IL-1 is regarded as a major mediator of chronic inflammatory joint diseases, including rheumatoid arthritis in man, adjuvant arthritis in rats and collagen-induced arthritis in mice. However, conclusive experimental evidence for the crucial role of IL-1 in the development of joint destruction has not been presented as yet. In the present study, we investigated the effect of a neutralizing monoclonal mouse antibody against mouse IL-1β (IgG1 isotype) on the development and progression of collagen-induced arthritis in DBA/1 mice. The antibody was injected intraperitoneally 3 times a week, either from day 3 or from day 21 after primary immunization, to day 60.*

In the positive control group an arthritis incidence of 80% was observed after 60 days. The injection of a control antibody of the same isotype did not influence the incidence of arthritis, whereas injection of anti-IL-1β from day 21 reduced the arthritis incidence to about 30%. Injection of anti-IL-1β starting at day 3 totally prevented both the development of arthritis and the associated increase of the acute phase protein serum amyloid P (SAP). Anti-collagen antibody titers, which increased significantly after immunization, were not influenced by the injection of anti-IL-1β antibodies, in spite of the suppressive effect on arthritis development. Joint destruction in the arthritic animals, as measured by X-ray scoring, was significantly influenced towards normalization in the animals treated with anti-IL-1β antibodies.

Taken together, our results present clear evidence for the involvement of IL-1β in the development and progression of collagen arthritis in mice.

Key words: collagen-induced arthritis, interleukin-1, monoclonal antibody, rheumatoid arthritis, acute phase response.

Introduction

Type II collagen-induced arthritis in rodents is regarded as one of the best animal models for rheumatoid arthritis and has been reported to have a number of characteristics in common with rheumatoid arthritis in man. Both diseases share the humoral and cellular immunological responses to type II collagen, although these responses are much more consistent in CIA compared to RA (1-4). In addition, MHC class II linkage (2, 5, 6) and a pronounced acute phase reaction (7-10) are characteristically observed in both diseases and the synovial inflammation of collagen-induced arthritis, as analyzed by histology and immunohistochemistry, is quite similar to the situation in active stages of RA (11, 12).

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Abbreviations used in this report: CIA: type II collagen-induced arthritis; collagen II: bovine type II collagen; ELISA: enzyme-linked immunosorbent assay; IL-1: interleukin-1; IFN-γ: interferon-γ; mAB: monoclonal antibody; MTT: thiazolylblue tetrazoliumbromide; SAP: serum amyloid P component; TNF-α: tumor necrosis factor α.

In the development and progression of both CIA and RA the pro-inflammatory activities of various cytokines such as IL-1α and IL-1β or TNF-α have been implicated (13-16). At present, it is not clear which member of the IL-1 family of monokines, IL-1α or IL-1β, plays the predominant role in the evolution of chronic inflammatory and arthritic diseases. With the availability of neutralizing antibodies to various cytokines in sufficient amounts during the last few years, it became possible to define the role of those cytokines in the pathophysiology of arthritic diseases.

In the present study, we investigated the effect of a neutralizing monoclonal antibody to murine IL-1β on the development of collagen-induced arthritis in DBA/1 mice. Our aim was to study whether the systemic neutralization of IL-1β exerted an effect on joint destruction, on the associated acute phase response, and on the humoral response to heterologous collagen.

Materials and methods

Materials. Keyhole limpet hemocyanin (KLH), rabbit anti-mouse SAP and goat anti-mouse IgG, peroxidase-linked, was obtained from Calbiochem (San Diego, USA). Donkey anti-rabbit IgG, horseradish peroxidase-linked antibody, was procured from Amersham Buchler (Buckinghamshire, UK). Recombinant murine IL-1α was obtained from British Biotechnology (Abingdon, UK).

Male DBA/1 H-2q mice, age 6 to 8 weeks, were used for the experiments.

Preparation and in vitro testing of monoclonal mouse anti-mouse IL-1β antibodies. Recombinant mouse IL-1β was expressed in *E. coli* using standard techniques. The hybridoma cell line producing the monoclonal anti-IL-1β antibody (1400.24.17) was generated by fusing spleen cells of a BALB/c mouse which had been repeatedly immunized with a conjugate of recombinant mouse IL-1β and KLH using described methods (17). The antibody was purified from ascites fluid by precipitation with ammonium sulfate and ion exchange chromatography. The control antibody 1226.31.3 against levoprotilin was generated identically.

IL-1 activity was determined by proliferation of D10S cells using the MTT protocol (18). Briefly, 2 x 10^4 cells were cultured in the presence or absence of the test antibody plus recombinant mouse IL-1β for 45 hours. The final volume was 200 µl. 100 µl were removed and replaced with 25 µl of an MTT (3-[4, 5 dimethylthiazol-2-yl]- 2,5-diphenyltetrazolium bromide; thiazolylblue) solution (5 mg/ml). After incubation for 2 hours at 37° C, 100 µl of a solution of 20% (w/v) sodium dodecylsulfate in DMF/water (1:1, ref. 19) were added to dissolve the crystals. The plates were read at 540 nM.

Preparation of fetal bovine collagen type II. A fetal calf was obtained from the slaughterhouse and the articular cartilage was dissected and extracted with a papain solution, essentially as described by Strawich and Nimni (20). All subsequent steps were performed at 4°C.

The viscous residue was further extracted with 0.45 M sodium chloride, followed by 0.5 M acetic acid. Acid soluble collagen II was precipitated with 2.7 M sodium chloride and redissolved in 0.5 M acetic acid. After centrifugation, the clear solution was dialysed against 20 mM sodium phosphate to precipitate type II collagen. Solubilization in acetic acid and precipitation in 2.7 M sodium chloride was repeated, and after resolubilization and extensive dialysis in 50 mM acetic acid, the type II collagen was lyophilized and stored at -80°C . The quality of the collagen II preparation was judged by electrophoresis on 5% polyacrylamide gels and the preparations were found to be essentially pure, except for trace amounts of type I and type IX collagen.

Arthritis induction and animal treatment. One volume of type II collagen, dissolved in 10 mM acetic acid (4 mg/ml), was emulsified in an equal volume of complete Freund's adjuvant (Sigma) by repeated passage through a nearly closed stopcock mounted between two 1 ml syringes. The final emulsion remained stable as a drop on the surface of water for more than 5 minutes. Male DBA/1 mice, 6 to 8 weeks of age, were immunized intradermally with 50 µl of the collagen emulsion at the base of the tail (100 µg type II collagen/mouse). The day of primary immunization corresponds to day zero in the figures. Three weeks later (corresponding to day 21), a booster injection of the same composition and amount was given at the contralateral side of the tail base.

All animal manipulations, other than the antibody treatment, were performed under isoflurane anesthesia (Forene®, Abbott, USA). Monoclonal anti-IL-1β antibodies were diluted in 0.9% pyrogen-free saline and injected three times per week intraperitoneally (100 µg/mouse per injection). Control animals were injected either with saline alone or with a non-cytokine monoclonal antibody of the same isotype (IgG1, mAb 1226.31.3), directed against an anti-depressant (anti-levoprotilin).

For the clinical scoring, arthritic symptoms were carefully examined three times a week and animals showing any sign of arthritis (swelling, redness of paws or limping) were considered arthritic. Blood was collected under deep anesthesia by orbital bleeding on day 21 and at the end of the experiment on day 60, before final asphyxiation in CO_2. For X-ray analysis, the animals were placed in a ventral position on Kodak X-Omat MA films and exposed with 28 kV, 125 mAs at a distance of 45 cm from the X-ray device (Mammomat, Siemens).

Assessment of X-ray photographs. Eighteen joints per paw were evaluated using a binocular microscope. Arthritis signs were loss of bone walls, loss of metaphyseal bone density or destruction of the joint architecture. Each joint was given a rating of 0 (non-arthritic) or 1 (arthritic). A score of 1 meant that at least one side of the articulation showed a clear loss of the bone wall contour or a clear loss of metaphyseal bone density. The theoretical maximum score was 72 per animal (all joints in all four paws affected). All X-ray photographs were evaluated at least twice in a blinded fashion by one investigator.

Determination of serum amyloid P component (SAP) by ELISA. SAP plasma levels were determined by a solid phase enzyme-linked immunoassay. The ELISA was performed essen-

tially as described by Serban and Rordorf-Adam (21). Briefly, the wells of polystyrene microtiter plates were coated with trinitrophenylated keyhole limpet hemocyanin (TNP-KLH), then incubated with SAP-containing samples. The wells were sequentially incubated with rabbit anti-mouse SAP antiserum and horseradish peroxidase-linked donkey anti-rabbit IgG conjugate. Purified mouse SAP was used as a standard. The ELISA plates were read at 490 nM in a computer-driven ELISA reader (Canberra Packard).

Determination of anti-collagen antibodies. 96-well microtiter plates were coated with type II collagen dissolved in sodium bicarbonate buffer, pH 8.3 (25 µg/ml). The sera were added at a 1:1000 dilution and bound mouse immunoglobulin was detected with peroxidase labeled goat antimouse IgG antiserum, essentially as described by Gosslau and Barrach (22).

Statistical analysis. Statistical analysis was performed using the InStat computer program (GraphPad™, San Diego, USA). The data are expressed as the mean ± SEM. Ten identically treated animals were used per group. The statistical significance between groups was calculated using the non-parametric Mann-Whitney U-test. For paired samples, the Wilcoxon signed-ranks test was used.

Results

Inhibition of D10S cell proliferation with monoclonal anti-ILβ antibody

The capacity of the monoclonal anti-IL-1β antibody 1400.24.17 to inhibit the proliferation of the murine T-cell line D10S was investigated. Figure 1 shows that about 50 ng/ml of monoclonal anti-IL-1β were required to half-maximally inhibit the proliferation induced by a constant concentration of murine IL-1β (100 pg/ml). In cultures without the addition of IL-1β, the antibody had no influence on the proliferation of D10S cells. Furthermore, the antibody did not interfere with the proliferation of D10S cells in response to human IL-1α (data not shown). Unspecific toxic effects of the antibody on the viability of D10S cells could thus be excluded.

The cross-reactivity of the monoclonal anti-IL-1β antibody with murine IL-1α was further investigated. The specificity of the monoclonal antibody 1400.24.17 for IL-1β was demonstrated by the ability of mouse IL-1β to efficiently compete with labeled IL-1β for binding to a limiting amount of antibody (Fig. 2). In contrast to IL-1β, IL-1α did not show any inhibition of binding. These results clearly demonstrate that mAB 1400.24.17 is specific for murine IL-1β, without crossreacting with mouse IL-1α.

Effect of the monoclonal anti-IL-1β antibody on the development and progression of collagen-induced arthritis in DBA-1 mice

Taking into account the *in vitro* neutralizing capacity of the antibody 1400.24.17, we injected 100 µg of monoclonal anti-IL-1β 3 times per week. Two different treatment schemes were selected, starting antibody treatment either at day 3 after primary immunization or at day 21, a time point where the early immunological reactions were already completed but where the arthritis was not yet manifest.

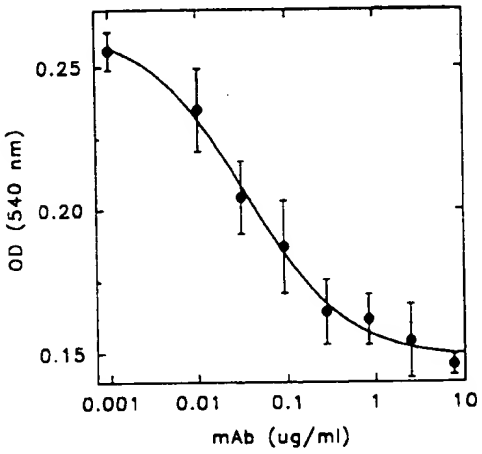

Fig. 1. Inhibition of IL-1-dependent proliferation of D10S cells by mAB 1400.24.17. 2 x 10^4 D10S cells were cultured in the presence or absence of monoclonal anti-IL-1β antibody and recombinant mouse IL-1β (100 pg/ml) for 45 hours. MTT solution was added and after incubation for 2 hrs at 37°C the crystals were dissolved and the plates were read at 540 nm.

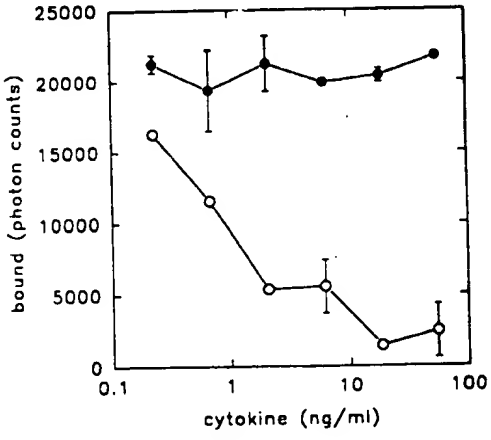

Fig. 2. Inhibition tests with mAB 1400.24.17 and mouse IL-1α or IL-1β. The monoclonal antibody 1400.24.17 (1.6 ng/ml) was incubated for 18 hrs at 4°C with varying concentrations of mouse IL-1α (●) or mouse IL-1β (○) in the presence of a constant amount of chemiluminescently labeled mouse IL-1β (1 ng/ml). Separation of bound from non-bound was achieved by incubation with paramagnetic particles carrying anti-mouse IgG immunoglobulins. After washing, the bound fraction was determined in a luminometer.

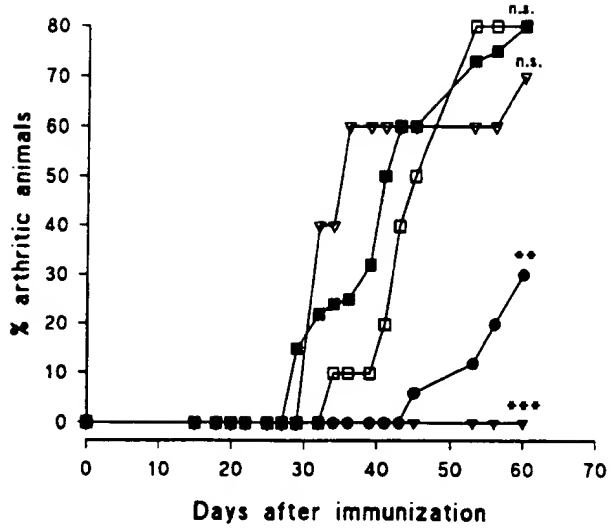

Fig. 3. The effect of anti-IL-1β antibodies on arthritis incidence: clinical scoring. CIA was induced in DBA/1 mice at day 0 (primary immunization) by an intracutaneous injection of bovine type II collagen in Freund's complete adjuvant. At day 21 the animals received a booster injection. Arthritis development was monitored 3 times a week (swelling, redness of paws, limping). Animals with any positive signs, irrespective of the severity, were regarded as arthritic, .
■ untreated control; ∇ saline; ❑ control antibody; ● anti-IL-1β (day 21 to end); ▼ anti-IL-1β (day 3 to end). Statistical analysis was performed using Fisher's test with the following limits: n.s: $p > 0.05$; ** $p < 0.01$; *** $p < 0.001$.

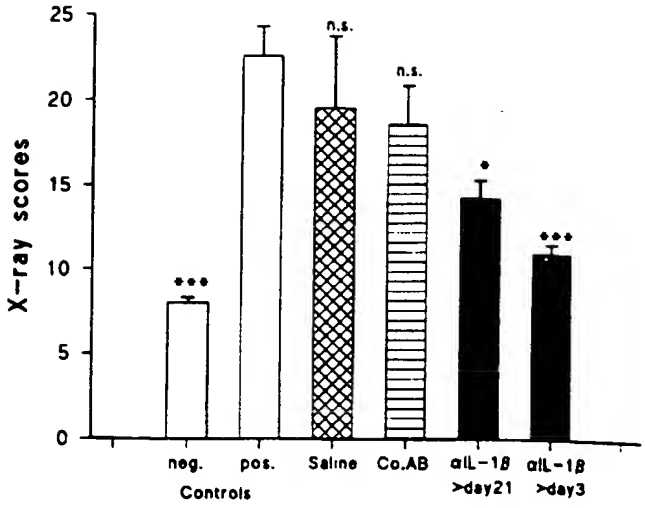

Fig. 4. The effect of anti-IL-1β antibodies on joint destruction: X-ray scoring. At day 60, the animals were sacrificed and X-ray pictures were taken as described in Materials and methods. The X-rays were evaluated using a binocular microscope in a double-blinded fashion. The theoretical maximal score was 72 (all 18 joints of all paws affected). Results are expressed as means ± SEM.
Open bars: controls (neg.: no arthritis induced; pos.: arthritic control); cross-hatched bars: saline injections, starting at day 3; horizontally striped bars: control antibody starting at day 3; solid bars: anti-IL-1β starting at day 21 (left) or day 3 (right). n.s.: not significant; * $p < 0.05$; *** $p < 0.001$ (Mann-Whitney U-test).

Figure 3 shows the clinical incidence of arthritis after the selected treatments. Arthritis developed in the untreated animals of the positive control group at day 30 and constantly increased until the end of the experiment at day 60, resulting in 80% arthritic animals. Injections of saline or control antibody did not significantly influence the time course or incidence of arthritis (n.s., $p > 0.05$). In contrast, treatment with anti-IL-1β antibodies starting at day 21 significantly reduced the arthritis incidence to about 30% ($p < 0.01$). In addition, a clear retardation of arthritis development was observed, with first clinical disease manifestations around day 50. Neutralization of IL-1β with the monoclonal antibody starting on day 3 after immunization totally prevented the occurrence of arthritis ($p < 0.001$). The animals in the negative control group (not immunized) did not develop any signs of arthritis (data not shown).

Effect of anti-IL-1β on joint destruction by X-ray scoring

Collagen-induced arthritis in mice results in the destruction of the joint architecture through the loss of cartilage and the demineralization of bone. The pro-inflammatory and catabolic activities of IL-1 have been implicated in these processes.

X-ray scoring of the joints of collagen-arthritis mice was performed at day 60. In Figure 4 it can be seen that established arthritis was associated with an increased number of affected joints. However, the scores in arthritic animals never reached the theoretical maximum of 72, as most of the arthritic animals only had one or two affected paws. Neither the injections of saline, nor the injection of control antibody resulted in a significant effect. In contrast, anti-IL-1β antibodies, given from day 21 up to the end of the experiment, reduced the joint destruction score significantly. When given from day 3 after immunization, the antibody was even more effective in ameliorating joint destruction ($p < 0.001$), although complete protection of joint destruction was not achieved, as compared to the negative control group.

Effect of anti-IL-1β on plasma levels of serum amyloid P (SAP)

SAP is one of the major positive acute phase proteins in the mouse and has been shown to increase during the development of arthritis in the CIA model (7, 21, 23).

Figure 5 shows the SAP levels in serum taken at day 21 (open bars) and at day 60 (solid bars). The SAP levels at day 60 were found to be significantly increased in arthritic animals (240 ± 103 μg/ml)) but were rather low in non-

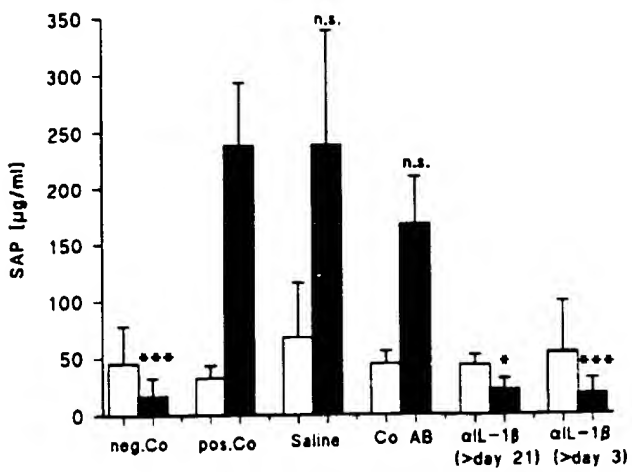

Fig. 5. The effect of anti-IL-1β antibodies on the acute phase response. Comparison of serum SAP levels at day 21 and day 60. Blood was collected by orbital bleeding at day 21 (open bars) and at day 60 (solid bars) for the determination of SAP. Serum SAP levels were measured by ELISA. The results are expressed as means ± SEM. n.s.: not significant; * $p < 0.05$; *** $p < 0.001$. The differences in serum SAP between days 21 and 60 for the various treatment groups were statistically analyzed using the Wilcoxon test for paired samples.

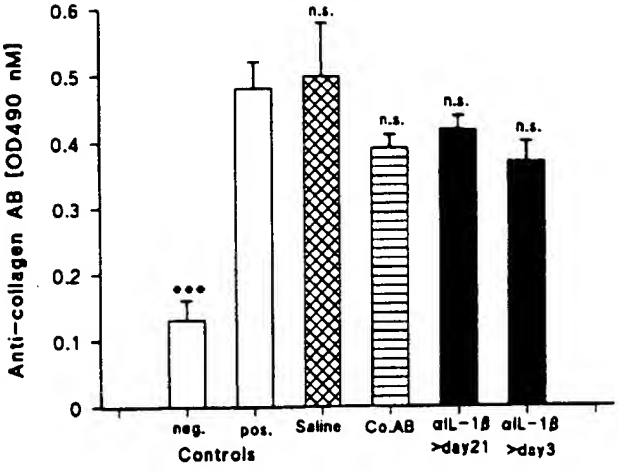

Fig. 6. The effect of anti-IL-1β antibodies on anti-collagen IgG antibody levels. CIA was induced as specified in the legend to Figure 3. On day 60, blood was collected by orbital bleeding under deep narcosis and the serum was prepared. Anti-collagen antibody titers (IgG) were determined by ELISA.
Results are expressed as means ± SEM. The symbols and confidence limits are the same as in Figure 4.

arthritic animals (17.2 ± 15 µg/ml, $p < 0.001$). Neither injections of saline nor injections of control antibody significantly reduced these elevated SAP levels. In contrast, injection of anti-IL-1β from day 21 or from day 3 onwards reduced the SAP levels (21 ± 9.1 µg/ml and 18.2 ± 13.8 µg/ml, respectively). The SAP levels of the latter group (day 3) were not statistically different from the levels of non-arthritic animals ($p = 0.13$, Mann-Whitney U-test).

SAP plasma levels at day 60 were further compared to those at day 21. Figure 5 shows that SAP levels in immunized animals at day 21 were not different from those of the animals in the negative control group, indicating that the acute-phase reaction in the animals is not observed before the development of arthritis. In arthritic animals, a highly significant increase in serum SAP levels was found between days 21 and 60 ($p < 0.01$). A significant increase was also observed in animals that had been treated with saline ($p < 0.01$) or with control antibody ($p < 0.05$). In contrast, mice that had been treated with anti-IL-1β antibodies starting at day 21 or at day 3 after immunization did not show an increase in serum SAP levels between days 21 and 60. The animals treated with anti-IL-1β antibodies instead showed a tendency toward decreased SAP levels, comparable to the animals of the negative control group (difference in SAP levels between days 21 and 60 not significant). These results demonstrate that the injection of anti-IL-1β antibodies totally prevented the arthritis-associated acute-phase response.

Effect of anti-IL-1β treatment on the humoral response to heterologous type II collagen

In the final set of measurements we investigated the humoral response to heterologous collagen, which has been found to result in high levels of circulating antibodies to type II collagen in CIA, first of the IgM isotype, later in the disease of the IgG isotype (1, 2, 24).

As shown in Figure 6, anti-collagen antibodies (IgG) increased significantly after immunization with type II collagen (day 60 after immunization). The titers of anti-collagen antibodies were not significantly influenced by the injection of either saline or control antibodies. Interestingly, injections of anti-IL-1β antibodies, starting either at day 21 or at day 3, had no significant effect on anti-collagen antibody titers, in spite of their suppressive effect on the other parameters of arthritis.

Discussion

In the present study we show that the evolution of collagen-induced arthritis in mice is suppressed by treatment of the animals with a neutralizing monoclonal antibody to IL-1β. The clear suppressive effect of the treatment with this antibody, which is directed only against IL-1β, was somewhat unexpected. The interleukin-1 family consists of two members, IL-1α and IL-1β, which are functionally indistinguishable and possess a wide spectrum of pro-

inflammatory properties, including bone demineralization, cartilage destruction, acute phase protein induction and fever induction (25).

Although IL-1α and IL-1β are the products of distinct genes, they bind to the same cell surface receptors and share various biological activities. While IL-1β is mainly secreted, IL-1α exists in a membrane-associated form that has been implicated in the antigen-presenting capacities of cells of the monocyte/macrophage lineage (25, 26). Our neutralizing antibody to IL-1β did not neutralize IL-1α, but nevertheless resulted in a clear and almost complete suppression of joint destruction in collagen-induced arthritis in mice and completely prevented the associated acute phase response. These results suggest an important role of IL-1β in the pathophysiological disturbances seen in collagen-induced arthritis, and perhaps also in different chronic inflammatory joint diseases, including rheumatoid arthritis in man.

Evidence for the involvement of cytokines in the development of collagen-induced arthritis has been presented recently, both by the application of recombinant cytokines and by the neutralization of cytokine activity with antibodies. Killar and Dunn (14) described the potentiation of collagen-induced arthritis in mice upon the systemic administration of recombinant IL-1β and concluded that this effect may be due in part to an augmentation of the immune response to heterologous collagen.

A similar potentiation of disease was described after the systemic application of TNF-α (15) or IL-1β (16) by osmotic minipumps or by the intra-articular injection of TNF-α or TGF-β (27), although in another report a protective effect of TGF-β was described (28). Suppressive effects on collagen arthritis were reported in studies using antibodies directed against the T-cell receptor αβ framework (29, 30) and after the subcutanous application of interferon-γ. The latter treatment was associated with a significant suppression of the serum anti-collagen antibody response (31).

Due to their various pro-inflammatory activities, including the induction of tissue-degrading enzymes in cartilage, IL-1β and TNF-α have been regarded as major mediators in the connective tissue destruction associated with chronic inflammatory joint diseases (32-34). The administration of neutralizing anti-TNF antibodies prior to disease onset significantly reduced paw swelling and histological severity without reducing the incidence of arthritis or the level of circulating anti-type II collagen IgG (35). Although in that study the levels of monoclonal antibodies administered were comparable to those of our study, we found a clear and almost complete suppressive effect on the incidence of arthritis when anti-IL-1β antibodies were injected prophylactically. This difference in efficiency might indicate that IL-1β is a predominant mediator for the development of arthritis, the neutralization of which is more favourable in terms of the prevention of arthritic disease. Nevertheless, it is not possible to predict which cytokine actually plays the dominant role in the development of arthritic diseases, due to the fact that the neutralization of one cytokine by the injection of antibodies might exert potent negative feedback effects on the entire cytokine system (25).

However, Piguet *et al.* (36) recently reported that the neutralization of TNF by either anti-TNF antibodies or soluble TNF receptors arrested the evolution of collagen arthritis, as shown by histological analysis. This effect was seen when treatment was started 2 weeks after immunization, but was not observed when treatment was started 2 months after induction of the disease. Consistent with our findings, treatment with anti-TNF antibodies did not influence the production of anti-collagen antibodies. These results are in agreement with our observations, in the sense that the potency of neutralizing IL-1β is most effective when started early after immunization. We entirely agree with the conclusions drawn by Piguet *et al.* (36) that the antagonism of cytokines, when achieved in an early phase, can prevent the long-term evolution of collagen arthritis. It would be interesting to investigate whether the inhibition of IL-1β, in contrast to TNF-α, still exerts an alleviating effect on arthritis when given therapeutically in established arthritis, instead of prophylactically.

In several studies the role of IL-1 in the evolution of collagen-arthritis was investigated by either injecting IL-1 and monitoring aggravation of experimental arthritis (14, 16, 37), by neutralizing IL-1 with antibodies (38), or by injecting recombinant human IL-1 receptor antagonist, an endogenous inhibitor of IL-1 (39). Most of these studies presented evidence for the important role of IL-1 in the development and progression of arthritic diseases. However, discrimination between the relative contributions of the two IL-1 forms, IL-1α or IL-1β, could not be achieved with these approaches.

Nevertheless, in the study by van de Loo *et al.* (38) the authors identified the released IL-1 in synovial tissue to be primarily of the IL-1α subtype. In that study it was demonstrated that the cartilage proteoglycan loss could be efficiently prevented by a mixture of neutralizing antibodies directed against both IL-1α and IL-1β. The finding of primarily IL-1α in synovial tissue contrasts with our results, inasmuch as we describe in this paper an almost complete protective effect of anti-IL-1β antibodies in mouse collagen-induced arthritis. One explanation for this discrepancy might be the stimulus used to induce the arthritis, i.e. heterologous collagen in our study and mBSA in the study by van de Loo *et al.* Another explanation might be differences in the specificity and/or affinity of the antibodies used. From our results we conclude that IL-1β is the mediator

predominantly responsible for the pathophysiological disturbances in collagen arthritis, including joint destruction and the concomitant acute phase reaction, although a secondary effect on IL-1α expression by negative feedback inhibition was not excluded specifically in our study.

The humoral response to heterologous collagen has been regarded as an absolute prerequisite for the development of arthritis in this animal model. Moreover, the rise in anti-collagen IgG antibodies has been shown to occur concurrently with the development of arthritic disease (40, 44). In addition, passive transfer of arthritis with anti-collagen antibodies has been achieved (41-43). These results were taken as an indication that the response to heterologous collagen is correlated with the evolution of arthritis. However, in this study we clearly show that the suppression of arthritis development by the application of an anti-IL-1β antibody occurs without an effect on anti-collagen antibody titers. Similar effects, i.e. alleviation of arthritis without suppression of anti-collagen antibody titers, have been described after the injection of anti-TNF antibodies (35, 36). In contrast, injection of anti-IFN-γ antibodies resulted in a significant modulation of collagen-arthritis which was associated with a pronounced suppression of the serum anti-collagen antibody response (31).

In accordance with published data (35, 36), our results suggest that the alleviation of arthritis is not necessarily reflected by a concomitant suppression of the humoral response to heterologous collagen, but can occur without reduction of anti-collagen antibody titers. This finding might be taken as an indication that the antibody response to collagen, although an absolute prerequisite for arthritis to develop, is not correlated to the severity and progression of the disease. In addition, these findings argue for an important role of cell-mediated immune responses in the pathophysiology of arthritic diseases, which are presumably negatively influenced by the inhibition of pro-inflammatory cytokines such as IL-1β or TNFα.

Taken together, the protective effect of the inhibition of the pro-inflammatory cytokine IL-1β in this model supports the concept that potent IL-1 and/or cytokine antagonists might supply us with new and hopefully more effective therapeutics for the treatment of rheumatoid arthritis and, perhaps, other autoimmune diseases.

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